

AD \_\_\_\_\_

GRANT NUMBER DAMD17-94-J-4294

TITLE: University of Kansas Cancer Center Breast Tissue and Serum Repository Core Facility

PRINCIPAL INVESTIGATOR: Jonathan J. Li, Ph.D.

CONTRACTING ORGANIZATION: University of Kansas Medical Center  
Kansas City, Missouri 66160-7700

REPORT DATE: October 1997

TYPE OF REPORT: Annual

PREPARED FOR: Commander  
U.S. Army Medical Research and Materiel Command  
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;  
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

PRO QUALITY INSPECTED

19980220 096

# REPORT DOCUMENTATION PAGE

*Form Approved  
OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY <i>(Leave blank)</i>	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
	October 1997	Annual (23 Sep 96 - 22 Sep 97)	
4. TITLE AND SUBTITLE		5. FUNDING NUMBERS	
University of Kansas Cancer Center Breast Tissue and Serum Repository Core Facility		DAMD17-94-J-4294	
6. AUTHOR(S)			
Jonathan J. Li, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)		8. PERFORMING ORGANIZATION REPORT NUMBER	
University of Kansas Medical Center Kansas City, Kansas 66160-7700			
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012			
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION / AVAILABILITY STATEMENT		12b. DISTRIBUTION CODE	
Approved for public release; distribution unlimited			
13. ABSTRACT <i>(Maximum 200)</i>			
The Division of Etiology and Prevention of Hormonal Cancers, Kansas Cancer Institute (KCI), has further developed in the third year of U.S. Army Medical Research and Development support a Breast Tissue and Serum Repository Core Facility (BTSR) to facilitate and foster breast cancer-related research at KCI and other research institutions in the Southern Plains States. To date, the BTSR has collected multiple malignant, nonmalignant, and normal breast tissue specimens, as well as serum, lymphocyte, and plasma specimens from consenting surgical patients. The collection and cataloging of endometrial and ovarian malignant and nonmalignant tissues and blood progresses. Approval received for collection of human tumor specimens from prostate, colon, thyroid, pancreas, lung, liver, and testicular cancers and corresponding normal tissues, serum, and lymphocyte samples. Initial collection will be restricted to endocrine-associated cancers. For each patient specimen, whether blood or tissue, a personal health history form has been completed. In addition, physician records of each patient are available if the information contained therein is needed by investigators. Patient confidentiality is strictly maintained, and patients' identities are not available to users of the BTSR Core Facility. A committee, comprising clinical and basic science faculty, reviews proposals for basic science and clinical studies.			
14. SUBJECT TERMS		15. NUMBER OF PAGES	
Breast Cancer		20	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

## FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

       Where copyrighted material is quoted, permission has been obtained to use such material.

       Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

       Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

       In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

✓ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

       In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

       In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

       In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

  
Mallika Li  
PI - Signature      Date  
10/22/97

Grant #DAMD17-94-J-4294  
Kansas Cancer Institute Breast Tissue  
and Serum Repository Core Facility  
PI: Jonathan J. Li, Ph.D.

## TABLE OF CONTENTS

<b>Introduction</b> .....	2
<b>Body</b> .....	2
<b>Results</b> .....	6
<b>Conclusions</b> .....	8
<b>Appendix:</b>	
KCI-BTSR Committee on Human Tissue Specimen .....	10
Usage	
Lymphocyte and Serum Separation .....	11
Revised Protocol for Breast Biopsies with Infiltrating	
Carcinoma	12
Tissue Summary Table .....	13
Blood Products Summary Table .....	14
Repository Inventory .....	15

## **INTRODUCTION**

The cause(s) of breast cancer and the means to predict who will develop it are currently not well understood. Understanding of either or both is an essential step to successful prevention of this prevalent disease in the future. Similarly, there is a paucity of knowledge related to early detection of breast cancer, because screening procedures, while improving, do not allow detection of breast cancer at the earliest and most curable stages. The development of the BTSR Core Facility at the KCI-KUMC is an important step to address these issues at this institution.

Development of the BTSR is highly relevant to expansion and augmentation of breast cancer research, including clinically-related and basic, at the Kansas Cancer Institute (KCI) and University of Kansas Medical Center (KUMC). The BTSR's purpose is to facilitate investigator-initiated research to perform correlation studies on the incidence of possible premalignant and malignant breast lesions with genetic and variable biomarkers (e.g., receptors, hormones, cellular proteins, protooncogenes, and tumor suppressor genes, etc.); and to assess the presence of potential carcinogens.

A focus of the Division of Etiology and Prevention of Hormonal Cancers (DEPHC) is to assist, complement, and expand existing, ongoing programs and to develop new programs in molecular biology and molecular cytogenetics in breast cancer research at KCI. A central emphasis of this Division is that hormones, particularly estrogens and progestin, play a critical role in breast tumor causation, progression, and dependency. Hormonal involvement in breast cancer etiology at the cellular and molecular level is not well understood and requires elucidation.

## **BODY**

### **I. Background**

After a delayed start as detailed in our 1995 report, the KCI-BTSR has been operational for 29 months and continues its excellent progress. Drs. Jonathan Li and Walter Imagawa continue as Director and Associate Director, respectively, Ms. Leslie Hudson continues as Biologist II, and Ms. Julianne Heaston continues as secretary.

## II. Experimental Methods

### Tissue Samples

Tissue samples for the KCI-BTSR are acquired from patients who have breast biopsies, lumpectomies, mastectomies, or breast reduction surgery, and also from women who undergo hysterectomy and/or oophorectomy for malignant and nonmalignant conditions. Ms. Leslie Hudson, the BTSR biologist, acquires the daily surgical schedule for all breast surgeries and is present in the Surgical Pathology Laboratory during the processing of the breast specimens. These are handled in a timely fashion in order to preserve the tissues appropriately. The breast tissue, normal, abnormal, and neoplastic, is placed on a frozen cutting board provided by the BTSR. The breast tissue specimens are delivered to the Surgical Pathology Laboratory within 10 min. A certified pathologist immediately evaluates the tumor, and a frozen section is prepared for diagnosis. The pathologist then cuts tumor/normal tissue specimens for the repository biologist if sufficient sample is available.

If there is sufficient tissue sample, one portion is allocated for frozen sections. Tissue samples destined for frozen section are covered with tissue-embedding medium in a cryomold, then placed in an airtight polypropylene container, labeled with a proper bar-code label (specimen-specific identification number--please see below), and immediately snap-frozen in a N<sub>2</sub> container before storage in the BTSR freezer. The remaining tissue sample is similarly labeled and snap-frozen in a polypropylene container.

Each specimen is assigned a unique six-digit specimen-specific identification number, which is assigned sequentially, with biopsy tissue, healthy adjacent tissue, and serum for a particular patient assigned the same number. All tissue aliquots derived from the same tissue are assigned the same six-digit number. This six-digit specimen-specific identification number is shown on the bar-code with which the biologist labels each container and slide.

A Surgical Pathology requisition form is computer generated by the Surgery Department and accompanies the specimen when it is delivered to the Pathology Laboratory. Information included on this form consists of hospital patient identification number, surgeon's name, patient's name and age, date of surgery, and site of specimen. In addition, Surgical Pathology personnel write the Surgical Pathology identification number on the requisition form, and the surgical pathologist measures the tumor before it is divided, indicating the size of the tumor. The repository biologist records the repository specimen-specific identification number on the requisition form. The BTSR biologist makes a copy of this form in the Pathology Laboratory and takes it to the BTSR along with the specimens. These data will eventually be entered into the BTSR database.

The following tests are routinely carried out on all malignant breast biopsy samples at KUMC (see protocol, p. 12):

- (1) estrogen and progesterone receptor analysis;
- (2) immunostaining for p53, HER-2/neu, and cathepsin;
- (3) ploidy analysis by flow cytometry or image analysis;
- (4) actual Surgical Pathology analysis, including a thorough analysis of tumor characteristics, histological type, histological grade, size, etc.

BTSR personnel can retrieve the results of all these tests as soon as they are available and enter the information into the BTSR database, as described below in Cataloging and Storage. Results from test (1) above are obtained from the Clinical Laboratory and test (4) results from the Flow Cytometry Laboratory, while those of the remaining tests are obtained from the Surgical Pathology Department.

### **Serum Samples**

Blood samples from both women having breast surgery and women at the KCI High Risk Breast Clinic will be submitted to the BTSR. The procedure described below is followed for each group of women.

Three days before a patient is scheduled to have breast surgery, she is required to go to the Outpatient Laboratory to have her blood drawn for various presurgical tests. It is the BTSR biologist's responsibility to secure the schedule of these visits in advance from the surgeons' scheduling nurse and to advise the Outpatient Laboratory to draw two extra vials of blood from each of these patients for the BTSR. The BTSR biologist is stationed in the Outpatient Laboratory at the time of each of these appointments to be sure that this extra blood is drawn and to label the blood vials with the proper outpatient laboratory labels, which include the patient's name and hospital patient identification number.

In addition, the BTSR biologist gives the patient consent forms for donating blood to the BTSR, asking the patient to sign these and to complete the Personal Health History questionnaire described in detail below under Storage and Cataloging. After the patient completes the questionnaire, the BTSR biologist writes the six-digit specimen-specific identification number on the upper right-hand corner of the front page of the questionnaire.

Women who are considered at high risk for breast cancer are eligible to participate in the KCI High-Risk Breast Clinic. In general, eligible women include those between 30 and 55 years of age who have at least one of the following conditions: a first-degree relative who has had breast cancer, or, in herself, precancerous mastopathy or prior node-negative breast cancer in one breast.

The High-Risk Breast Clinic is located at the KU Cancer Center Comprehensive Outpatient Diagnostic and Treatment Center. During each patient's first visit to the clinic, blood is drawn for various medical tests. The BTSR biologist is responsible for securing the schedule of these visits in advance and advising the clinic to draw one extra vial of blood from each new patient for the Serum Repository. The identical procedure described above for securing the blood and completed questionnaire from breast surgery patients at the Outpatient Laboratory is also followed for new patients seen at the High-Risk Breast Clinic.

When blood specimens are received at the BTSR, the biologist processes the blood before the specimens are cataloged and stored in the freezer. After spinning down the reamed whole clotted blood in a refrigerated centrifuge, she removes the vial cap and, with a sterile pipette, divides the sera into 1.5-ml aliquots in the polypropylene containers. Each container is then labeled with the proper bar-code label and snap-frozen. Three times a day, the labels are scanned and the appropriate data entered into the Biopsy Serum Table, the Reduction Mammoplasty Serum Table or the High Risk Serum Table, depending on the source of the serum.

The specimen-specific number on the bar-code label will have been assigned to all specimens obtained. The six-digit identification number is identical to the number assigned to the tissue specimen for the same patient, when applicable.

Similar procedures have been developed for the collection of tissues and blood from gynecological patients. The questionnaire used for breast patients has been modified for these patients.

### **Lymphocyte Samples**

The BTSR has the capacity to separate and freeze lymphocytes from peripheral blood when a special request is received. Blood will be collected in heparin-containing tubes. A 10-ml tube is necessary. Preferably, within two hours after blood collection, the procedure detailed on p. 11 should be followed.

After all serum and lymphocytes are separated and labeled, the BTSR biologist then stores the tissue and serum samples in the freezer and records all data regarding storage location in the Location Table of the database. These data include specimen identification number and sample

location, including freezer shelf, box and cubicle number. This will allow the BTSR staff to locate all specimens quickly and easily.

### **Storage and Cataloging**

When a tissue sample is received at the KCI-BTSR, specimen bar codes are scanned into the Biopsy Table, the Healthy Adjacent Table, or the Reduction Mammoplasty Table of the Repository Database, as appropriate; the unique hospital patient identification number, the date that the specimen is received by the BTSR, the hospital of origin, the total amount of tissue, the surgical date, and all other data shown on the surgical requisition form that accompanies each specimen are then keyed in.

All specimen-specific and patient-specific data are maintained in the computerized Repository Database Management system, developed by the Program Database Leader using FoxPro for Windows, a database management software package. FoxPro is a relational database system that allows for various files in the system to be linked by means of key fields. In the Repository Database, the key fields are the unique specimen number and a combination of the hospital patient identification number and the Surgical Pathology identification number. This combination serves as a unique patient identifier. Any or all of the tables within the database are linked using these three fields.

When a patient questionnaire is delivered to the Repository, it is initially labeled with the appropriate bar code. The six-digit identification number matches those of the other specimens for the same patient. The questionnaire labels are then scanned and the data entered into the Demographic/Life Style Table. Responses to this questionnaire will be extremely valuable to many research investigators who will be using the BTSR breast specimens. The data requested include demographic, physical, and lifestyle information. Specifically, questions concern age, racial/ethnic background, marital status, religion, weight, height, education, occupation, family income, family history of breast cancer, age at first period, and menopausal, childbirth, lactation and alcohol history. To maintain confidentiality, all questionnaires are filed and locked up in a secure location after the data are entered into the database.

## **RESULTS**

During the past year, the repository has substantially increased its inventory of breast tissues and blood products. The total number of breast, endometrial, and ovarian tissue specimens with accompanying blood samples and histology blocks is summarized on p. 13. The repository now has in storage 66 specimens of malignant breast tissue, 90 specimens of nonmalignant breast tissue (e.g., fibroadenoma, fibrocystic, etc.), and 17 normal specimens from breast reductions. The repository

has also begun collecting endometrial and ovarian tissues and blood products from patients undergoing gynecological surgery. Fifteen malignant endometrial and 51 nonmalignant tissue specimens, as well as 13 malignant and 62 nonmalignant ovarian tissue specimens, are currently in storage.

The repository has begun collecting plasma in addition to serum and lymphocytes. The collection of these specimens is summarized on p. 14. The total serum and lymphocyte samples for breast tissues have increased, and now 137 and 127 specimens, respectively, are stored and available, as well as 40 plasma samples. For surgical patients from whom tissue is not available, blood is still collected for the repository. However, it is sometimes unavailable due to patient refusal. Total blood samples for endometrial and ovarian tissue now number 99.

On pp. 15-17 appears an example of the KCI-BTSR inventory method, indicating the bar coding of samples and sample location in the repository freezer.

Proposals have been solicited from KUMC and outside investigators for use of specimens from the repository. When received, proposals are reviewed by the KCI-BTSR Committee on Human Tissue Specimen Usage for approval. The following investigators are currently approved for specimen use:

Investigator	% Estimated Use	Research Support
Jonathan J. Li, Ph.D. (KUMC) Sara Antonia Li, Ph.D. (KUMC)	5%	NCI 5 R01 CA 58030-05
Walter T. Imagawa, Ph.D. (KUMC)	10%	NCI CA 68414-01 USAMRMC BC960604
Gregory Reed, Ph.D. (KUMC)	10%	Dept. of Pharmacology institutional funds
Carol Fabian, M.D. (KUMC)	15%	NCI PO1 CA 72094 NCI UO1 CA 72296 NCI MAA NCI CN 45593-32 NCI N01 CN 65024-32
Dr. Lin Tao (U. Missouri-Kansas City)	10%	NIH KS-34647

Leslie Heckert, Ph.D. (KUMC)	10%	Kansas Cancer Institute institutional funds
Tsuneo Suzuki, M.D., Ph.D. (KUMC)	10%	NIH P01 CA 54474
Eric Elsinghorst, Ph.D. (U. Kansas-Lawrence)	5%	Dept. of Microbiology institutional funds

## CONCLUSIONS

The repository has successfully established itself with a growing inventory and database. In addition, the collection of tissue specimens from gynecological cancers has expanded the utility of the repository to a broader range of investigators. We have furthermore received human subjects approval to collect lung, liver, colon and testicular tissues, which will also add to the usefulness of the repository.

Currently, the Breast Tissue and Serum Repository (BTSR) Core Facility is collecting serum, lymphocytes, and normal and tumor breast tissue from patients. The BTSR has extended its collection capabilities to other estrogen-related cancers of the endometrium and ovary. At present, the BTSR has received human subjects approval to collect additional human material from patients at other organ sites, including prostate, colon, liver, testicular, pancreas, and lung. Over the final year of BTSR funding, the focus will be to collect human specimens of endocrine-related cancers. While our main objective remains to collect normal and breast tumor tissues, as well as serum and lymphocytes, from these patients, these other tissues and body fluid materials will be collected at minimal cost to the BTSR and supported by the Departments of Surgery and Pathology. These human tissues have been requested by a number of investigators, and the BTSR is the logical and appropriate resource to store, classify, and record these human materials for research use.

### **Future Goals**

1. Increase collection of breast tissue by outreach to other local hospitals.
2. Continue to call for breast, serum, and lymphocyte proposals from investigators at KUMC (Kansas City), Kansas State University (Basic Cancer Center), and KUMC (Wichita) (Women's Health Institute). The multidisciplinary review committee (p. 10) will continue to review these proposals.

Grant #DAMD17-94-J-4294  
Kansas Cancer Institute Breast Tissue  
and Serum Repository Core Facility  
PI: Jonathan J. Li, Ph.D.

3. Expand collection of human tumor specimens to prostate, colon, thyroid, pancreas, lung, liver, and testicular cancers and corresponding normal tissues, as well as corresponding serum, lymphocyte, and plasma samples. Since a number of KUMC investigators have research interests in cancers at these organ sites, it seems useful to expand cancer research studies at KUMC by making these tumors available to all interested investigators, for which we have now received human subjects approval.

Grant #DAMD17-94-J-4294  
Kansas Cancer Institute Breast Tissue  
and Serum Repository Core Facility  
PI: Jonathan J. Li, Ph.D.

## APPENDIX

## **KCI-BTSR Committee on Human Tissue Specimen Usage**

William Jewell, M.D. - Surgeon (breast), Professor and Director, Kansas Cancer Institute  
Jonathan J. Li, Ph.D. - Director, BTSR Core Facility, Professor  
Sara Antonia Li, Ph.D. - Hormonal Carcinogenesis Researcher, Associate Professor  
Janet Woodroof, M.D. - Pathologist, Assistant Professor  
Walter Imagawa, Ph.D. - Breast Cancer Researcher and Associate Director, BTSR Core Facility,  
Assistant Professor  
Carol Fabian, M.D. - Medical Oncologist (breast), Professor  
Paramjit Bhatia, M.D. - Chief, Surgical Pathology, Associate Professor

## Serum Separation

Collect blood in 10ml Serum Separator Tubes.

Allow to sit at room temperature for 1 - 2 hours and follow this procedure:

1. Centrifuge at 1500 rpm for 15 minutes at 4°C;
2. Aliquot 1.5 mL serum into each bar code-labelled, chilled vial, noting in database amount in each vial.
3. Freeze at -80°C.

## Lymphocyte Separation - CPT

Collect blood in a CPT Vacutainer Tube containing heparin additive. Gently invert tube 8 times.  
Within two hours of blood collection, follow this procedure:

1. Centrifuge at 1500 x g in a horizontal rotor (swing-out head) for 15 minutes at room temperature;
2. Collect top layer of plasma and aliquot into cryovials, noting aliquot amounts;
3. Collect opaque layer of lymphocytes and monocytes and transfer to 15 ml centrifuge tube containing approximately 5 ml Hank's Solution, mix by gently inverting tube;
4. Centrifuge at 250 x g (may increase if necessary) for 10 minutes at room temperature;
5. Discard supernatant;
6. Resuspend pellet in 0.5ml of Hank's Solution;
7. Determine cell count using Crystal Violet (Stain 0.05mL cell solution with 0.45mL Crystal Violet and vortex for 20 sec.); Count number of stained cells in hemocytometer (determine total cells);
8. Put cells in bar code-labelled vial;
9. Freeze at -80°C.

### Materials needed:

15 ml capped centrifuge tubes  
Hank's Solution  
Crystal Violet  
5mL pipets  
1mL pipets  
200uL micropipet

INVASIVE MAMMARY CARCINOMA PROTOCOL

Tumor size <1cm in greatest diameter:

- 1) Scrape for ploidy by image analysis, take to image room (Marilyn or Julie) <sup>a</sup>
- 2) Scrape for flow cytometry (call Bill Justice -3876) <sup>b</sup>
- 3) Order ER/PR, MIB-1, p53, HER-2/neu, cathepsin immunostains (give req to Julie) <sup>c</sup>

Tumor size 1 to 1.5cm in greatest diameter:

- 1) Scrape for ploidy by image analysis, take to image room (Marilyn or Julie) <sup>a</sup>
- 2) Scrape for flow cytometry (call Bill Justice -3876) <sup>b</sup>
- 3) Send 0.5cm piece of tumor for ER/PR EIA (call metabolic lab) <sup>d</sup>
- 4) Order MIB-1, p53, HER-2/neu, cathepsin immunostains (give req to Julie) <sup>c</sup>

Tumor size 1.5 to 2.0cm in greatest diameter:

- 1) Scrape for ploidy by image analysis, take to image room (Marilyn or Julie) <sup>a</sup>
- 2) Send 0.5cm piece of tumor for ER/PR EIA (call metabolic lab) <sup>d</sup>
- 3) Place a 0.5cm piece of tumor into RPMI for flow cytometry (call Bill Justice -3876) <sup>e</sup>
- 4) Order MIB-1, p53, HER-2/neu, cathepsin immunostains (give req to Julie) <sup>c</sup>

Tumor size >2.0cm in greatest diameter:

- 1) Scrape for ploidy by image analysis, take to image room (Marilyn or Julie) <sup>a</sup>
- 2) Send 0.5cm piece of tumor for ER/PR EIA (call metabolic lab) <sup>d</sup>
- 3) Place a 0.5cm piece of tumor into RPMI for flow cytometry (call Bill Justice -3876) <sup>e</sup>
- 4) Order MIB-1, p53, HER-2/neu, cathepsin immunostains (give req to Julie) <sup>c</sup>
- 5) Submit 0.5cm of tumor to Oncotech (in Oncotech media).
- 5) Submit at least 0.5cm of tumor to the breast tumor bank (Leslie Hudson).

<sup>a</sup> Scrape for ploidy by image analysis. Scrape tumor with surgical blade and place material in the middle of a superfrost slide; using another superfrost slide, gently smear the material across the slide, and let air dry. Take the slide and a copy of the requisition to the image analysis room. Page Marilyn Davis if you have questions (6098).

<sup>b</sup> Scrape tumor for flow cytometry. Use a small amount of pressure when scraping. After each of 6 scrapes (3/tumor slice) immerse the scalpel blade in RPMI medium and shake the blade to dislodge the cells. Place the sample in the refrigerator with a copy of the requisition, and call Bill Justice (ext 3876).

<sup>c</sup> Order ER/PR, MIB-1, p53, HER-2/neu, and cathepsin immunostains on a paraffin block (block in which the tissue opposite the frozen section is submitted). Do not order on frozen section block unless it is the only block with tumor.

<sup>d</sup> Place a 0.5cm piece of tumor into a labeled plastic bag and freeze immediately in liquid nitrogen. Fill out green requisition requesting "ER/PR". Call metabolic lab (7020) to send out to Roche.

<sup>e</sup> Place a 0.5cm piece of tumor into RPMI for flow cytometry. Call Bill Justice (ext 3876).

**Breast Tissue and Serum Repository**  
**Specimen Update 10/17/97**

**Tissues**

**BREAST TISSUE**

	Malignant	Non-Malignant	Normal
<b>Total Samples</b>	<b>66</b>	<b>90</b>	<b>17</b>
<b>Blood Samples*</b>	<b>44</b>	<b>32</b>	<b>1</b>
<b>Histology Blocks</b>	<b>40</b>	<b>51</b>	<b>10</b>
<b>Complete Sets</b>	<b>26</b>	<b>25</b>	<b>1</b>

\*Blood not obtained due to patient decline to consent.

**ENDOMETRIAL TISSUE**

	Malignant	Non-Malignant
<b>Total Samples</b>	<b>15</b>	<b>51</b>
<b>Blood Samples</b>	<b>13</b>	<b>46</b>
<b>Histology Blocks</b>	<b>11</b>	<b>25</b>
<b>Complete Sets</b>	<b>10</b>	<b>23</b>

**OVARIAN TISSUE**

	Malignant	Non-Malignant
<b>Total Samples</b>	<b>13</b>	<b>62</b>
<b>Blood Samples</b>	<b>13</b>	<b>57</b>
<b>Histology Blocks</b>	<b>8</b>	<b>41</b>
<b>Complete Sets</b>	<b>8</b>	<b>40</b>

## Blood Products

	Breast		
	Total Samples	Malignant	Non-Malignant
Serum Samples	137	89	46
Lymphocyte Samples	127	80	44
Plasma Samples	40	26	14

	Endometrium		
	Total Samples	Malignant	Non-Malignant
Serum Samples	60	14	49
Lymphocyte Samples	58	14	47
Plasma Samples	12	6	7

	Ovary		
	Total Samples	Malignant	Non-Malignant
Serum Samples	69	15	57
Lymphocyte Samples	66	15	54
Plasma Samples	17	5	12

Grant #DAMD17-94-J-4294  
**Example of Breast Tissue Inventory**

Kansas Cancer Institute Breast Tissue

and Serum Repository Core Facility

PI: Jonathan J. Li, Ph.D.

**Malignant and Non-Malignant Tissue**

**Breast**

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location
100000	5/12/95	72	2	1A1.1-2 malignant tissue
100001	5/17/95	76	1	1A1.3 malignant tissue
100009	6/5/95	46	1	*1A1.4 malignant tissue
100011	6/5/95	28	7	1A1.5-6, 1B1.1-5 malignant tissue
100018	8/16/95	43	13	1A1.7-19 (mastectomy w/non-malignant tissue)
100020	8/16/95	52	1	1A1.20 malignant tissue
100022	9/8/95	47	5	1A1.21-25 (mastectomy - only 1A1.25 contains malignant tissue)
000003	9/8/95	71	9	1A1.26-34 (mastectomy w/non-malignant tissue)
000004	9/1/95	60	3	1A1.35-37 malignant tissue
000005	9/20/95	46	5	1A1.38-42 non-malignant tissue
000007	9/1/95	37	1	*1B4.2 non-malignant
000011	9/25/95	60	2	1A1.43-44 malignant tissue
000013	10/18/95	77	1	1A1.51 non-malignant tissue
000014	11/3/95	83	2	1A1.45-46 non-malignant tissue (male)
000016	11/8/95	42	1	1A1.47 non-malignant tissue
000017	10/24/95	43	1	1A1.48 malignant tissue
000018	11/21/95	42	4	1A1.69-72 non-malignant tissue
000019	10/26/95	52	1	1A1.49 malignant
	11/27/95		14	1A1.55-65, 1B2.1-3 non-malignant
000023	11/21/95	31	1	1A1.50 malignant
000024	12/1/95	26	1	1A1.52 non-malignant
000027	12/8/95	33	1	1A1.53 non-malignant
000029	12/18/95	59	1	1A1.54 malignant
000030	12/20/95	22	1	1A1.73 non-malignant
000031	12/20/95	58	2	1A1.66 malignant, 1A3.23 non-malignant
000032	12/29/95	55	1	1A1.67 malignant
000033	2/19/96	36	1	1A1.68 malignant
000036	12/22/95	83	1	*1A2.37 malignant
000039	1/26/96	44	4	*1A2.38-40 non-malignant, 1B4.3 malignant
000040	1/29/96	47	9	1A2.4-8 (L) non-malignant, 1B2.4-7 (R) non-malignant (presence of i.s.possible)
000041	11/29/95	42	1	1A1.74 non-malignant
000057	3/27/96	53	1	1A2.9 non-malignant
000060	11/8/95	49	2	1A1.76-77 malignant
	2/26/96		7	1A1.78-81, 1A2.1-3 non-malignant
000063	3/13/96	58	1	1A1.75 non-malignant
000064	4/1/96	56	4	1A2.13-16 non-malignant (presence of malignant possible)
000066	3/25/96	25	1	1A2.10 non-malignant
000070	3/13/96	76	2	1A2.11-12 malignant
000074	3/25/96	42	1	1A2.25 non-malignant
000078	4/15/96	44	2	1A2.17-18malignant & non-malignant
000080	3/27/96	34	1	1A2.32 malignant
000081	4/22/96	83	1	1A2.19 non-malignant
000083	5/1/96	49	3	1A2.20,21-22 malignant
000091	1/19/96	64	1	1A2.26 malignant
000095	5/22/96	51	1	1A2.23microcalcifications
000096	5/20/96	33	1	1A2.27 non-malignant
000104	6/10/96	45	4	1B3.6-9 non-malignant
000111	4/3/96	55	1	1A2.24 malignant
000117	7/1/96	69	2	1A2.61-62 malignant and non-malignant

## Example of Breast Serum Inventory

Grant #DAMD17-94-J-4294  
 Kansas Cancer Institute Breast Tissue  
 and Serum Repository Core Facility  
 PI: Jonathan J. Li, Ph.D.

### Blood Serum

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location	Sample Class
100000	5/12/95	72	4	1C1.12-15	Breast Cancer
100001	5/17/95	76	3	1C1.25-27	Breast Cancer
100002	5/19/95	51	2	1C1.1-2	Fibrocystic
100003	5/22/95	59	3	1C1.3-5	Fibrocystic
100004	5/22/95	68	3	1C1.6-8	Breast Cancer
100005	5/22/95	54	3	1C1.9-11	Breast Cancer
100006	5/24/95	57	3	1C1.16-18	Breast Cancer
100009	6/5/95	46	3	1C1.22-24	*Breast Cancer
100011	6/5/95	28	3	1C1.19-21	Breast Cancer
100012	7/10/95	78	3	1C1.28-30	Breast Cancer
100013	7/19/95	56	3	1C1.35-37	Breast Cancer
100014	7/12/95	60	3	1C1.32-34	Breast Cancer (7 primaries)
100015	7/19/95	43	2	1C1.38-39	Breast Cancer
100016	8/11/95	39	3	1C1.41-43	Fibrocystic
100017	8/30/95	68	3	1C1.52-54	Breast Cancer
100019	8/16/95	42	3	1C1.48-50	Breast Cancer
100020	8/16/95	52	1	1C1.51	Breast Cancer
100022	9/8/95	47	4	1C1.55-58	Breast Cancer
000001	9/6/95	45	3	1C1.65-67	Fibrocystic
000002	9/8/95	51	2	1C1.70-71	Fibrocystic
000003	9/8/95	71	1	1C1.72	Breast Cancer
000004	9/1/95	60	4	1C2.43-46	Breast Cancer
000005	9/13/95	46	3	1C1.75-77	Breast Cancer
000006	9/13/95	70	2	1C1.78-79	Breast Cancer
000010	9/22/95	52	2	1C1.80-81	Fibrocystic
000011	9/25/95	60	3	1C2.3-5	Breast Cancer
000012	10/2/95	64	3	1C2.7-9	Fibrocystic
000013	10/18/95	77	3	1C2.11-13	Fibrocystic
000015	11/8/95	44	3	1C2.16-18	Breast Cancer
000016	11/8/95	42	3	1C2.19-21	Fibrocystic
000017	10/24/95	43	3	1C2.22-24	Breast Cancer
000018	11/21/95	42	2	1C2.25-26	Breast Cancer
000019	11/27/95	52	3	1C2.31-33	Breast Cancer
000020	11/17/95	44	1	1C2.34	Fibrocystic
000021	11/17/95	53	3	1C2.35-37	Fibrocystic
000022	8/9/95	65	3	1C1.61-63	Fibrocystic
000023	11/21/95	31	3	1C2.39-41	Breast Cancer
000025	12/6/95	49	3	1C2.49-51	*Breast Cancer
000026	12/6/95	59	3	1C2.53-55	*Breast Cancer
000027	12/8/95	33	3	1C2.57-59	Fibrocystic
000028	11/22/95	76	2	1C2.75-76	Breast Cancer
000029	12/18/95	59	3	1C2.66-68	Breast Cancer
000030	12/20/95	22	3	1C2.61-63	Fibrocystic
000031	12/20/95	58	3	1C2.69-71	Breast Cancer
000032	12/29/95	55	3	1C3.11-13	Breast Cancer
000033	9/22/95	36	3	1C2.77-79	Breast Cancer
000034	1/10/96	72	3	1C3.3-5	Breast Cancer
000035	12/27/95	46	3	1C3.7-9	Breast Cancer
000037	1/22/96	65	2	2C1.2-3	Endometrial Cancer
000038	1/24/96	42	3	2C1.5-7	Ovarian Cancer
000039	1/26/96	44	3	1C3.22-24	*Breast Cancer
000040	1/29/96	47	3	1C3.26-28	Breast Cancer

## Example of Breast Lymphocyte Inventory

### Lymphocytes

Bar Code	Date of Procedure	Age of Patient	# Vials	Sample Location	Sample Class
100014	7/12/95	60	2	1C1.31,40	Breast Cancer
100016	8/11/95	39	1	1C1.44	Fibrocystic
100017	8/14/95	68	1	1C1.45	Breast Cancer
100019	8/16/95	42	1	1C1.47	Breast Cancer
100020	8/16/95	52	1	1C1.46	Breast Cancer
000022	8/9/95	65	1	1C1.60	Fibrocystic
100022	9/8/95	47	1	1C1.59	Breast Cancer
000001	9/6/95	45	1	1C1.64	Fibrocystic
000002	9/8/95	51	1	1C1.68	Fibrocystic
000003	9/8/95	71	1	1C1.69	Breast Cancer
000004	9/1/95	60	1	1C2.42	Breast Cancer
000005	9/13/95	46	1	1C1.73	Breast Cancer
000006	9/13/95	70	1	1C1.74	Breast Cancer
000010	9/22/95	52	1	1C2.1	Fibrocystic
000011	9/25/95	60	1	1C2.2	Breast Cancer
000012	10/2/95	64	1	1C2.6	Fibrocystic
000013	10/18/95	77	1	1C2.10	Fibrocystic
000015	11/8/95	44	1	1C2.14	Breast Cancer
000016	11/8/95	42	1	1C2.15	Fibrocystic
000017	10/24/95	43	1	1C2.27	Breast Cancer
000018	11/21/95	42	1	1C2.47	Breast Cancer
000019	11/27/95	52	1	1C2.28	Breast Cancer
000020	11/17/95	44	1	1C2.29	Fibrocystic
000021	11/17/95	53	1	1C2.30	Fibrocystic
000023	11/21/95	31	1	1C2.38	Breast Cancer
000025	12/6/95	49	1	1C2.48	*Breast Cancer
000026	12/6/95	59	1	1C2.52	*Breast Cancer
000027	12/8/95	33	1	1C2.56	Fibrocystic
000028	11/22/95	76	1	1C2.81	Breast Cancer
000029	12/18/95	59	1	1C2.64	Breast Cancer
000030	12/20/95	22	1	1C2.60	Fibrocystic
000031	12/20/95	58	1	1C2.65	Breast Cancer
000032	12/29/95	55	1	1C3.10	Breast Cancer
000033	9/22/95	36	1	1C3.1	Breast Cancer
000034	1/10/96	72	1	1C3.2	Breast Cancer
000035	12/27/95	46	1	1C3.6	Breast Cancer
000037	1/22/96	65	1	2C1.1	Endometrial Cancer
000038	1/24/96	42	1	2C1.4	Ovarian Cancer
000039	1/26/96	44	1	1C3.21	*Breast Cancer
000040	1/29/96	47	1	1C3.25	Breast Cancer
000041	11/29/95	42	1	1C3.64	Fibrocystic
000042	1/30/96	42	1	2C1.8	Endometritis, adenomyosis
000043	1/30/96	34	1	2C1.12	Cervical Cancer
000044	1/31/96	46	1	2C1.16	Post-menopausal bleeding
000045	2/6/96	28	1	2C1.20	(squamous cell cancer of cx)
000046	2/7/96	54	2	2C1.23,61	non-malignant endometrium
000047	2/8/96	11	1	2C1.27	malignant ovary
000048	2/8/96	53	1	2C1.31	(ovarian cancer)
000050	2/16/96	55	1	2C1.35	in situ carcinoma endometrium
000051	2/16/96	37	1	2C1.39	(ovarian mass)
000052	2/21/96	49	1	2C1.43	non-malignant endometrium, non-malignant ovary
000053	2/27/96	72	1	2C1.47	*Prolapsed uterus